# THE ACTION OF THE VISIBLE SPECTRUM ON COMPLEMENT

ENRIQUE E. ECKER

*From the Department of Pathology, Western Reserve University, and Nela Research Laboratory, Cleveland, Ohio*

The inhibitory power of the invisible (ultraviolet) rays on complement has received extensive recognition. Recent studies by Baroni and Jonesco Mihaïesti,[1] Abelin and Stiner,[2] Courmont, Nogier and Dufourt,[3] Bovie[4] and Brooks[5] have definitely established this particular phase of photodynamic destruction of complement and amboceptor. Lundberg[6] recently found that the speed of photo-attenuation of complement markedly increases as attenuation proceeds. McCoy, Hill and Schmidt,[7] on the other hand, showed the protective action of the aromatic amino-acids (tyrosin and phenylalanin) for complement and amboceptor against the action of these rays. The bulk of this work has been done by direct exposure of serums to the ultraviolet rays varying the thickness of the exposed liquids, or by dilution ending with a simple complement titration. It would appear, then, that attempts to study the effect of the visible rays on complement and amboceptor are entirely lacking. In the present work the action of the visible part of the spectrum (rays of greater wave lengths) was studied to determine their effect on complement.

*Methods of Study.*—The source of light employed was a powerful 15 ampères tungsten filament lamp of 944 candles per square centimeter operating on 17 volts. The beam of light of this lamp was thrown through a set of projecting lenses and focused on the slit of a large Hilger's spectrograph containing a glass prism. The width of the slit was 1 mm. and its length 7.5 mm. The serums of guinea-pigs were exposed in the spectrum in a glass spectrum cell carrying exactly 1.5 c c of fluid and having a diameter of 4 mm. Care was taken not to fill the cell to capacity surpassing the upper or lower margins of the

Received for publication, June 12, 1922.

1 Compt. rend Soc. de biol., 1910, 68, p. 393.
2 Ztschr. f. Immunitätsf. u. Exper. Therap., 1913, 19, p. 1.
3 Compt. rend. Soc. de biol., 1913, 74, p. 1152.
4 Jour. Med. Res., 1918-19, 38, p. 355.
5 Ibid., p. 345.
6 Compt. rend. Soc. de biol., 1921, 85, p. 758.
7 Jour. Infect. Dis., 1919, 25, p. 335.

spectrum. At the side of the exposed serum the control half of the serum was kept in a small test tube wrapped in black paper. The average temperature of the laboratory was 25 C. and throughout the experiment the room was kept dark. The exposures were continuous and for varying periods of time. Serums (0.2 c c) were dried in air on glass slides and then exposed in a dried state. Dilutions of fresh serums were made up to 10% and exposed. In addition to this set of experiments, a trial was made to use the direct rays of the sun by means of a Fuss heliostat, focusing the image of the sun on the slit of the Hilger's spectrograph. So far exposures to the solar spectrum have been of short duration, 2.5 and 4.5 hours, respectively. As regards the loss of light, it may be said that altogether about 20% of the total light passes through the prism so that a considerable amount of light is lost by the use of the lenses and spectrograph. The spectrum was further divided into 2 and 3 regions and complements exposed to the rays of each region. The 2 regional divisions were: (a) reds, orange and yellow, and (b) green, blue and violet. The solar spectrum was divided in 3 regions because of the greater lengths of the red and infra red rays. These regions were: (a) infra reds and reds; (b) yellow and green, and (c) blue and violet. It was found impossible to divide the spectrum in more regions because of its size and the amounts of serums necessary for accurate titrations.

After the exposures, titrations were made by using an antisheep amboceptor cell system. Two units of amboceptor and 5% cell suspensions were used, the total volume of each being 0.25 c c. Complement was employed in quantities of 0.05, 0.075, 0.125, 0.150, 0.175, 0.200, 0.250, and 0.300 of 1 c c of a 10% dilution. Saline was added to balance. The time of incubation was 30 minutes at 37 C. in a water bath.

Table 1 presents the results of a series of exposures to the entire spectrum and for various periods of time. The serum used in these tests was undiluted.

The results of these experiments indicate clearly that 3 hours' exposure had practically no effect on the serum, while from 6 hours on the inhibitory action becomes visible and gradually increasing until the complement is practically destroyed after an exposure of 29 hours. Several exposures were made for periods less than 3 hours but with no effect.

Table 2 gives the results of exposures of diluted serum (10%) and also of dried serum. The diluted serum in this experiment was

the same as the one used in the first undiluted (3 hours) experiment. Whereas in the first experiment only 0.125 of 1 c c of the complement was necessary to produce complete hemolysis, the same serum exposed in a diluted state required 0.175 of 1 c c to produce complete hemolysis (3 hours). Nineteen hours' exposures entirely inhibited the activating power of 0.125 of 1 c c of the undiluted serum, and 0.25 of 1 c c of the same serum diluted was required to give slight hemolysis. It seems

TABLE 1

EFFECT OF THE ENTIRE VISIBLE SPECTRUM ON COMPLEMENT

| Amounts of Complement Used | 3 Hours | | 6 Hours | | 19 Hours | | 29 Hours | |
|---|---|---|---|---|---|---|---|---|
| | Exposed | Dark | Exposed | Dark | Exposed | Dark | Exposed | Dark |
| 0.05 c c | NH | SH | NH | NH | NH | NH | NH | NH |
| 0.075 c c | SH | ACH | NH | MH | NH | TH | NH | NH |
| 0.100 c c | ACH | CH | NH | CH | NH | SH | NH | NH |
| 0.125 c c | CH | CH | NH | CH | NH | MH | NH | SH |
| 0.150 c c | CH | CH | TH | CH | TH | ACH | NH | MH |
| 0.175 c c | CH | CH | SH | CH | SH | CH | NH | CH |
| 0.200 c c | CH | CH | MH | CH | MH | CH | NH | CH |
| 0.250 c c | CH | CH | MH | CH | ACH | CH | NH | CH |
| 0.300 c c | CH | CH | .... | .... | CH | CH | SH | CH |

CH, complete hemolysis; ACH, almost complete hemolysis; MH, marked hemolysis; SH, slight hemolysis; TH, trace hemolysis; NH, no hemolysis.

TABLE 2

EFFECT OF THE ENTIRE VISIBLE SPECTRUM ON DILUTED AND UNDILUTED COMPLEMENT

| Amount of Complement Used | Diluted | | | | Dried | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 Hours | | 19 Hours | | 24 Hours | | 48 Hours | |
| | Exposed | Dark | Exposed | Dark | Exposed | Dark | Exposed | Dark |
| 0.05 c c | NH | SH | NH | NH | NH | NH | SH | MH |
| 0.075 c c | SH | MH | NH | SH | NH | NH | MH | CH |
| 0.100 c c | MH | ACH | NH | MH | SH | SH | ACH | CH |
| 0.125 c c | MH | CH | NH | CH | MH | MH | CH | CH |
| 0.150 c c | ACH | CH | NH | CH | ACH | ACH | CH | CH |
| 0.175 c c | CH | CH | NH | CH | CH | CH | CH | CH |
| 0.200 c c | CH | CH | TH | CH | CH | CH | CH | CH |
| 0.250 c c | CH | CH | SH | CH | CH | CH | CH | CH |
| 0.300 c c | CH | CH | | | | | | |

evident, therefore, that diluted serum is distinctly more sensitive to the action of light than undiluted serum.

Dried serum, on the other hand, seems to be much more resistant to the action of these rays. Forty-eight hours' exposure only slightly modified the complementing power of the serums used, while 6 hour exposures were sufficient to attenuate normal undried serums. In table 3 the comparative effect of the rays of larger wave lengths to those of shorter wave lengths are tabulated.

This experiment shows clearly that the green-blue-violet end of the spectrum is considerably more active in the destruction of complement than the red-orange and yellow end. The experiment further demonstrates that the red-orange and yellow rays have some inhibiting action on complement. There was a demonstrable delay of hemolysis, and, as shown, 0.152 of 1 c c of exposed complement was necessary to secure complete hemolysis, while 0.075 of 1 c c of the same nonexposed serum sufficed.

TABLE 3

THE ACTION OF THE RAYS OF LARGER WAVE LENGTHS COMPARED TO THAT OF THE RAYS OF THE SHORTER WAVE LENGTHS ON COMPLEMENT. (EXPOSURE 19 HOURS)

| Amount of Complement | Reds, Orange, Yellow Regions | | Green, Blue, Violet Regions | |
|---|---|---|---|---|
| | Exposed | Dark | Exposed | Dark |
| 0.05 c c | MH | MH | NH | NH |
| 0.075 c c | MH | CH | NH | MH |
| 0.100 c c | ACH | CH | NH | ACH |
| 0.125 c c | CH | CH | NH | CH |
| 0.150 c c | CH | CH | NH | CH |
| 0.175 c c | CH | CH | TH | CH |
| 0.200 c c | CH | CH | SH | CH |
| 0.250 c c | CH | CH | MH | CH |

TABLE 4

DIVISION OF SOLAR SPECTRUM INTO THREE REGIONS AND THE EFFECT OF EACH REGION ON COMPLEMENT

| Amount of Complement | 2.5 Hours | | Control | | 4.5 Hours | | Control | |
|---|---|---|---|---|---|---|---|---|
| | Infra-red Red | Yellow-green | Blue-violet | Dark | Infra-red Red | Yellow-green | Blue-violet | Dark |
| 0.050 c c | NH | NH | NH | MH | NH | NH | NH | SH |
| 0.100 c c | NH | NH | NH | ACH | TH | TH | NH | CH |
| 0.125 c c | MH | SH | SH | CH | MH | MH | SH | CH |
| 0.150 c c | CH | CH | CH | CH | ACH | ACH | MH | CH |
| 0.175 c c | CH | CH | CH | CH | CH | CH | CH | CH |
| 0.200 c c | CH | CH | CH | CH | CH | CH | CH | CH |
| 0.250 c c | CH | CH | CH | CH | CH | CH | CH | CH |

In the following experiment the solar spectrum has been divided into three zones, the infra-reds and red, the yellow-green and the blue-violet zones. The light of the sun is rich in infra-reds and red rays, and it is for that reason that the study of the solar spectrum is of importance to determine the direct effect of these rays (table 4).

From the results, it can be seen that the red and infra-red rays have a slight inhibitory action on complement. The effect of these rays was mainly one of delay of reaction when compared with the control not exposed half of the serum. As stated, the effects of the solar spectrum deserve further study.

SUMMARY

The effect of the visible spectrum on complement is one of inhibition. Exposed serums do not produce hemolysis in the same unit of time as serums kept in darkness and may be even greatly reduced in their complementary action if the exposures have been sufficiently long. Hemolysis is distinctly delayed, and if the exposures have been made for a long enough period the effect produced is lasting. Red and infra-red rays delay the activating action of the complement. At the violet end of the spectrum inhibition is marked. Dried serums are more resistant to the action of light and only prolonged exposures are able to weaken the complement, while diluted serums are much less resistant. The decrease of complementary power after an exposure of 6 hours, for instance, does not continue after the radiation is terminated. The reaction, then, is not continuous. This confirms the work of Bovie and Brooks and of Huber [8] for exposures of rennin to ultraviolet rays. With continued exposure, however, attenuation markedly increases as pointed out by Lundberg.[6] The nature of light action on complement is still problematical. It appears as if it is a purely chemical change. Abelin and Stiner [2] state the action of light (ultraviolet) to be one of molecular change and give as an example the change of the poisonous yellow-phosphorus into nonpoisonous red-phosphorous. The action does not take place with absorption. Bovie showed that 20% of the cells protect complement from destruction, and Soret [9] has shown that most proteins exhibit an absorption band in the ultraviolet end of the spectrum and that solutions of tyrosin exhibit this phenomenon most markedly. Only tyrosin and phenylanin seem to show this property as reported by Kober.[11] Whatever the nature of photosensitiveness of complement, light inhibits and destroys complement, and its action can be antagonized by the use of a member of the aromatic-amino acid series, as shown most recently by McCoy, Hill and Schmidt. It remains for future work to determine the exact nature of this effect.

[8] Arch. f. Hyg., 1905, 54, p. 53.
[9] Arch. d. Sc. phys. et nat. Geneva, 1878, p. 322; 1883, p. 194.
[10] Jour. Biol. Chem., 1915, 22, p. 433.